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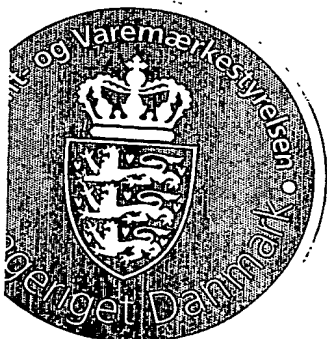
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A Method for Cell Implantation

Field of the invention

- 5 The present invention relates to a method for cell implantation or transplantation involving the use of an arthroscope or an endoscope. The method enables arthroscopic and/or endoscopic implantation of cells and is an easy, safe and cheap method.

Background of the invention

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- Mats Brittberg et al. have shown that a fibrin adhesive such as Tisseel® with, or without Growth hormone does not have a chondrogenic effect on immature chondrocytes, and it is therefore not suitable as a scaffold to promote repair of chondral or osteochondral defects (Brittberg, M, Sjögren-Jansson, Lindahl, A, Peterson, L, The influence of fibrin sealant on
- 15 Osteochondral Defect Repair in the Rabbit Knee, from Mats Brittberg's Thesis, Cartilage Repair. On cartilaginous tissue engineering with the emphasis on chondrocyte transplantation, Dep. Of Orthopedics, Institute of Surgical Sciences and Dep. Of Clinical Chemistry, Institute of Laboratory Medicine, Göteborg University, Sweden, 1996).
- 20 Lazovic and Messner (Lazovic, D, et al., Acta Ortop. Scand, 1993,64:583-586) examined the use of the fibrin adhesive (Tisseel®) in an attempt to improve the healing of transected anterior cruciate ligament in dogs, and found that ligaments, which were repaired using the adhesive, showed low range development of collagen versus simply suturing the ligament, which actually resulted in much higher content of collagen. They postulated that
- 25 fibrin adhesive chemically enhanced cell proliferation, but the repair tissue formed was of inferior quality. In Brittberg et al's experiments using Tisseel® for the repair of osteochondral defects in rabbits, they found that there was less repair tissue in the Tisseel® group, but the repair tissue was of the same fibrous-fibrocartilaginous quality regardless of whether Tisseel® was used or not used. They concluded that Tisseel® did
- 30 not improve the repair of osteochondral defect, whereas autologous or homologous fibrin clots used as support appeared to show beneficial effect sufficient to merit further *in vivo* exams.

- Further, Brittberg et al. found that experiments where chondrocytes were used together
- 35 with Tisseel® did not exhibit any toxic reaction, but the chondrocytes were only growing on the surface of the Tisseel® clot indicating that Tisseel® could be used as a barrier, such as for instance sealing the periphery of a cover such as for instance a periosteal

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graft as used in ACI. However experiments including homologous fibrin clots showed full in-growth of both rabbit and human chondrocytes, thus indicating that this type of clot showed a "scaffold-conductive" effect on the cells.

- 5 In experiments with Tisseel® plus injection of growth hormone (GH) did not reinforce any scaffolding effect, but would rather serve as a hemostatic barrier, whereas homologous (and autologous) fibrin appeared to promote infiltration of and growth of chondrocytes into the fibrin clot, thus indicating that this type of fibrin may be exhibiting a scaffolding effect. It is therefore possible that homologous (or autologous) fibrin may exhibit a growth
10 promoting effect on chondrocytes and an increased migration into the fibrin.

- The development of a suitable arthroscopic method for chondrocyte implantation and more specifically, autologous chondrocyte implantation, as well as for cell implantation methods – besides for the repair of chondral lesions – theoretically to be used for the
15 repair of osteochondritis, osteoarthritis, also called osteoarthrosis, and chondromalacia of patella, etc. would significantly bring the cost down, when compared to ACI performed during open knee surgery. Next, chondrocyte implantation and other cell implantation would render the ACI technology as well as other cell implantation technologies in the orthopedic field, more accessible in – besides knee joints, in other joints. Further, virtually
20 any orthopedic clinics that performs arthroscopic interventions, would gain access to using the ACI technology, and thereby more patients would be able to be treated with ACI in the repair of their cartilage defects (and later, when cell implantation for other orthopedic diseases becomes available, these techniques would also be subjected to development of arthroscopic guided treatment.

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Accordingly, there is a need for developing an arthroscopic method in order to enable a fuller utilization of the cell implantation technology in the field of orthopedic surgery.

- Methods using scaffold technologies of various forms, where the scaffold (with, or without
30 cells grown in the scaffold) is inserted into the defect, have suffered from difficulties in performing the cell implantation procedure solely guided by arthroscopy.

Detailed disclosure of the invention

- 35 The present invention meets the above-mentioned needs by providing a method for arthroscopic or endoscopic implantation of homologous or autologous cells into a defect of an animal body, the method comprising

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- i) application of a fluid to a cavity or surface containing the defect via arthroscope or endoscope to locate area of defect to be repaired or target area for cell transplantation ,
 - ii) application of the cells to the defect substantially simultaneously with a supporting material, acting as a binding scaffold, coagulating scaffold or gelating scaffold
 - 5 iii) mixing of the cells and the supporting material,
 - iv) solidification of the supporting material so that the defect or the target area is covered by a mixture of cells and supporting material, and
 - v) optionally, removal of the fluid from the cavity or surface via drainage or suction.
- 10 The fluid employed may be a liquid such as physiologically acceptable a sodium chloride solution, Ringer's solution or the like or it may be a gas such as e.g. sterile air comprising CO₂ in a concentration that is compatible with the tissue in question (e.g. a joint or an organ of the animal body; such as, e.g., a mammal like a human). Normally, a concentration of CO₂ of about 5% is suitable. Such fluids are normally used during
- 15 arthroscopy and endoscopy and are well known to a person skilled in the art.

In the present context the term "tissue" is used in a broad sense to cover soft tissue (organs etc.) as well as hard tissue (bones, joints etc).

- 20 The cells are homologous or autologous cells and are of a type that is suitable for use for repairing the defect in question. In the present context the term "homologous cells" is intended to mean that the cells are compatible with the tissue to which they are applied. The term "autologous cells" indicates that the cells are derived from the same subject to which the cells are applied. In the following e.g. the use of chondrocytes and/or
- 25 osteocytes are discussed. The cells applied are normally presented in a suspension, i.e. the cells are suspended in a suitable medium such as, e.g., Eagle medium (e.g. Eagle MEMHam F12) optionally containing serum (e.g. fetal calf serum or homologous or autologous serum) and/or growth factors, and the time of application, mixed with the supporting material.
- 30 As mentioned above, the cells are applied to the defect substantially simultaneously, preferably simultaneously, with a support material. The support material is a material that is capable of coagulating or solidifying upon application to the defect. Thus, the support material should - when it has solidified *in situ* - function as a tight cover that adhere to the
- 35 defect or the target area and should enable the cells to be maintained at the defect or target area. Furthermore, the cover has the function of protecting the treated defect or target area from the influence from the local environment.

In general, the supporting material in which the cells are mixed or dispersed is typically a coagulating, adhering, binding, gelating /or a sealant product. The supporting material may be diluted with a suitable medium before application through a suitable device to the defect or target area. The composition comprising the support material may contain suitable materials as e.g. fibrin, collagen type I, III, II, or the like, and for instance together with Insulin Growth Factor (IGF) and/or other growth factors.

It is important that the three different liquids (i.e. I) the medium with cells, and II) the composition comprising the supporting material. The flow of the supporting material enables the necessary mixing of the cells and material into the defect or target area. Alternatively or additionally, a flow of gas may be applied (e.g. also via the arthroscope) instead of the fluid applied in order to control the view of the defect area, or target area.

The invention encompasses the utilization of either implantation of cells simultaneously with a supporting material, into which the cells are mixed during the application, - in the first part of the invention - performed either under a fluid cover, using the general usage of an arthroscope where a fluid is used to keep the joint open for visualization, or as a second part of the invention in a combination with an arthroscope, which provides a certain pressure of guided sterile air such as for instance used in other endoscopes - for instance for abdominal exams, etc., where air is supplied under a certain pressure - such as for instance a combination of atmospheric air combined with CO₂, in a concentration well tolerated by the mixed cell/coagulating support material. In this second part of the invention, the "cover" used, under which the mixed cell/coagulation support material may be placed under the air pocket, where the pressure may be around 20 - 30 mm Hg.

The third part of the invention relates to a layer of a coagulating substance (support material) which is applied under either the fluid or under the inflated air, - this coagulating substance will be cell-free and consist of covering the mixed cell/coagulating support material.

This first part of the invention concerns the utilization of the presence of fluid, the possibility of changing the pressure of fluid in the joint (and possibly sterile air pressure) during a arthroscopically or endoscopically guided procedure combined with controlling the implantation of a liquid substance, mixed with cells, for instance such as chondrocytes for the repair of cartilage defects, by utilizing the pressure to keep the supporting material, for instance mixed with cells in place, for instance at the bottom of a cartilage defect or

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other areas of target, while the substance with – or without the cells solidifies and adhere to the defect. This method utilizes the difference in solubility of two substances, where a pressure applied to one type of fluid (or air gas) controls the localization of the other type of fluid substance, without mixing with the first type of fluid. In other words, the support material or the composition comprising the support material is not soluble in the fluid cover, i.e. the support material will be maintained in the application area (e.g. the bottom of a joint) where it solidifies and adheres to the application area. The simultaneous application of the cells enables the supporting material to incorporate or envelope the cells so that the cells are maintained at the application area as well.

10 This method combining the presence of fluid in the joint applied via the arthroscope for the implantation of the supporting material – and the resulting pressure applied, an increase in the pressure of the fluid during solidification of the liquid substance with or without cells, the decrease in the pressure and/or the removal of the pressure, when the solidification and adherence is sufficient to hold the implant in place in the defect is a novel technology, developed for the purpose of enabling the specialist to control the placement of the liquid substance while applying the substance with – or without cells, and to alter the pressure of the fluid (or if air gas is used to alter the pressure of the gas), as needed, without exceeding a pressure that would endanger implanted cells. The time for the solidification of a supporting material, which at the same time permits proliferation and migration of for instance chondrocytes when for instance applied to the supporting material without being mixed with the fluid added to control the viscosity through the application procedure. The ability of certain fibrins to allow proliferation and migration of chondrocytes is described by Mats Brittberg, as described above. However, this procedure done by Brittberg, is not performed as a "two phase" system, e.g. under fluid or gas used for visualization.

Another aspect of the invention is that the method described comprising placing a cell mixed in a supporting material under fluid or under air pressure also may be used for application of more than one cell type such as chondrogenic cells (chondrocytes/chondroblasts or other chondrogenic cells) and osteogenic cells such as osteoblasts, osteocytes, or other cells, etc. in order to enable a two step procedure, where the bottom part of an osteoarthritic lesion, for instance, initially is treated with a mix of osteogenic cells and supporting material performed under fluid and/or under air pressure, - next a mix of chondrogenic cells and supporting material are layered over the first cell/supporting material (layer 1) – under fluid and/or air pressure.

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Yet another aspect of the inventions is to deliver a hydroxyl apatite granulate (e.g., Bio-Oss, Geistlich Biomaterials, Wolhusen, Switzerland) either containing cultured osteogenic cells or delivered together with osteogenic cells – all in a mixture applied instantly together with supporting material under fluid and/or under air pressure in a defect such as an
 5 osteoarthritic defect in a joint. Next, a layer of a mixture of chondrogenic cells/supporting material is layered over the bottom layer consisting of hydroxyl apatite granulate.

In any the above described parts of the invention in which a mixture of cells and suitable supporting material, or cells, hydroxyapatite, and supporting material is placed – for
 10 instance during or via arthroscopic intervention, a cover layer consisting of a cell-free layer of fibrin as for instance Tisseel® biological glue (in which the chondrocytes for instance will not migrate into, which in this case will coagulate with little or no cells in the upper covering layer – will be placed.

15 The cover layer consisting of either fibrin such as the Tisseel® composite may be mixed with another protein (e.g., collagen (for instance collagen type I) as a soluble substance mixed with fibrin or with Tisseel®, in order to strengthen the cover which will face the surface of the joint tangential to the surrounding healthy cartilage. This may be done during arthroscopy for instance under fluid or air pressure.

20 The cover may also be a membrane, cut to the size of the defect (e.g., a collagen type I/III membrane) with the side, facing the cell/coagulation support material, coated with a fresh highly concentrated homologous or supporting material or with fresh Tisseel®, in order to prevent the cell/coagulation support material to allow cells into the cover, and at the same
 25 time fasten to the cell/coagulation supporting material. The membrane may also by itself adhere to the cell/coagulation supporting material.

When using an arthroscopic method it is therefore necessary to be capable of performing application of chondrocytes such as autologous chondrocytes together with a substance,
 30 where one is able to control the application of the chondrocytes in a substance that will remain (adhere) to the area in which the cells shall be kept, in spite of the presence of (sterile) physiological saline or nutrient medium in the joint, due to the use of an arthroscope as guidance for performing autologous chondrocyte implantation.

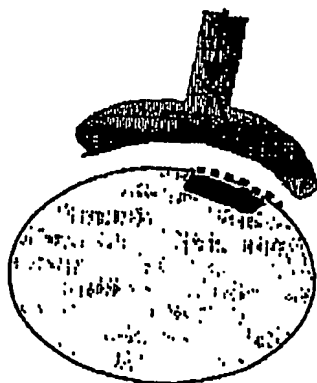
35 The method developed by the present inventor is considered novel, due to the new concept of placing a chondrocyte-containing substance at a controlled area in for instance a cartilage defect, such as a chondral defect, an osteochondral defect, or even when applying cells such as osteoblasts/osteocytes in a substance such as for instance

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hydroxyl-apatite, as a thin bottom layer in a joint suffering from osteoarthritis, and thereafter being able to apply a second layer of chondrocyte/substance as well as when applying cells through an endoscopic procedure to a target organ or target area. The substance shall be of a nature that at the same time is not toxic to the cells, and in case of chondrocytes, the substance applied under fluid, shall be promoting chondrocyte in-growth.

Different concentration levels of the suitable supporting material used for the mixture and variation of the coagulation, adhering, and/or gelating time as well as variation of the volume and concentration of cells to be mixed with the supporting material is part of this invention. Furthermore, cells and the supporting material may be mixed with adequate growth factors such as for instance IGF-1, which may help maintain differentiated chondrocyte morphology in fibrin (Fortier, LA, et al., Am. J. Vet. Res. 2002, 63(2) 301-305).

As described above an integral part of the invention is also to create a the above described cover either as a surface of fibrin or of Tisseel® or one of these agents applied together with another protein which may make a smooth surface with a relative stronger tensile strength (such as for instance soluble collagen), or possibly mixed with various constituents, such as for instance soluble collagen, and other substances providing a smooth surface of the repaired cartilage area, etc. by creating a spreading outlet that, when in use will enable the surgeon to apply the fibrin to a convex or a concave joint surface, in which there is a defect as shown in the following figure. During the arthroscopy a temporary perforated membrane may be placed stretching over the surface of the cartilage, lining and limiting the fibrin/cell mix and fibrin cover, when injected below the fluid or below an air pressure. The membrane shall be easily permeable for fluid used (or air inflated) during the arthroscopy. The membrane is then removed from one of the incisions when the fibrin/cell mix and the fibrin cover are coagulated in place.



The following examples are intended to illustrate the invention without limiting the scope in any way.

5 Example 1

A joint derived from an animal, such as for instance a knee joint from a pig or a horse or other animals are subjected to arthroscopy and fluid such as 0.9% sterile saline or a gas is filling up the joint to visualize the area targeted for the arthroscopic repair of the
10 area as for instance a cartilage defect in order to enable the application of the supporting material mixed with the cells in such a manner that the cell/supporting material does not mix with the fluid or gas applied via the arthroscope, and with a retained capability to adhere to the target area.

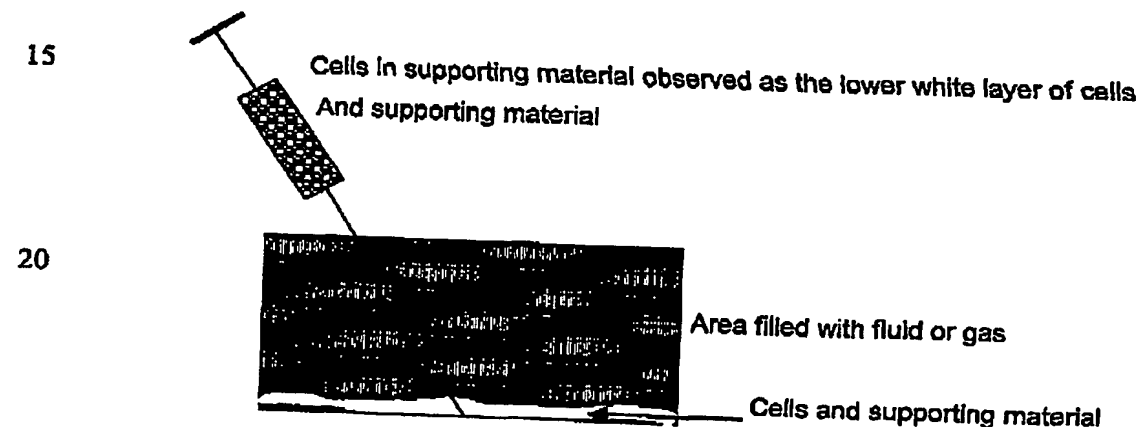


Figure 1. The syringe outlet containing the support material mixed with cells.

30 Alternatively, the cells and or the cells mixed or grown in a hydroxy-apatite suspension (e.g., Bio-Oss), may be delivered through the syringe, and be mixed at the outlet of the syringe into the area to be treated.

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Example 2

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In another example, the supporting material may be placed – under pressure from the other liquid, prior to the implantation of the cells. In this case, the supporting material may be placed below the other liquid for instance kept in place by the pressure exerted via the arthroscope until solidification. The cells are then injected at any time after the placement
5 of the liquid substance or after the solidification has occurred, by simply injecting the cells into the supporting material kept in place by the fluid pressure.

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Claims

1. A method for arthroscopic or endoscopic implantation of homologous or autologous cells into a defect of an animal body, the method comprising a step of
 - i) arthroscopic or endoscopic application of a fluid to a cavity or surface containing the defect
 - followed by the steps of
 - ii) application of the cells to the defect substantially simultaneously with a support material, the application being performed at the defect covered by the fluid,
 - iii) mixing of the cells and the supporting material,
 - iv) solidification of the supporting material so that the defect is covered by a mixture of cells and support material without any significant amount of fluid, and
 - v) optionally, removal of the fluid from the cavity or surface by drainage or suction.
2. A method according to claim 1, wherein the animal is a mammal such as a human.
3. A method according to claims 1-2, wherein the defect is a joint or bone defect.
4. A method according to claim 3, wherein the defect is a cartilage defect.
5. A method according to any of the preceding claims, wherein the cells are chondrocytes or osteoblasts.
6. A method according to any of the preceding claims, wherein the cells are of suitable origine for targeting a suitable tissue, where the visualization is done by an endoscope.
7. A method according to any of the preceding claims, wherein the cells are applied in the form of a suspension of cells in a suitable medium such as, e.g., a suitable growth medium optionally comprising one or more growth factors.
8. A method according to claims 5 - 7, wherein the application of the aqueous medium is simultaneously to the application of the cells and the supporting medium.
9. A method according to any of the preceding claims further comprising application of hydroxy apatite e.g. in the form of a hydroxy apatite granulate.

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10. Use of hydroxy apatite as culturing medium for cells such as osteoblasts/osteocytes.

11. Use of collagen solutions as culturing medium for the cells to be arthroscopically transplanted.

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12. Use of collagen solutions as culturing medium for the cells to be endoscopically transplanted.

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